

The Shared Core Resource as a Partner in Innovative Scientific Research: Illustration from an Academic Microscopy Imaging Center

**Douglas J. Taatjes¹, Prachi N. Ghule², Nicole A. Bouffard³, Kyra Lee⁴,
Nicole M. DeLance³, Mark F. Evans⁵, Donald L. Weaver⁵,
Nicholas Deakin⁶, Frances E. Carr⁷, Brian L. Sprague⁸, Gary S. Stein²,
Janet L. Stein²**

¹Department of Pathology and Laboratory Medicine, Microscopy Imaging Center, and University of Vermont Cancer Center, University of Vermont, Burlington, VT 05405, USA,

²University of Vermont Cancer Center and Department of Biochemistry, University of Vermont, Burlington, VT 05405, USA,

³Department of Pathology and Laboratory Medicine and Microscopy Imaging Center, University of Vermont, Burlington, VT 05405, USA,

⁴Department of Biochemistry, University of Vermont, Burlington, VT 05405, USA,

⁵Department of Pathology and Laboratory Medicine and University of Vermont Cancer Center, University of Vermont, Burlington, VT 05405, USA,

⁶Nikon Instruments Inc., Melville, NY, USA,

⁷University of Vermont Cancer Center and Department of Pharmacology, University of Vermont, Burlington, VT 05405, USA,

⁸University of Vermont Cancer Center and Department of Surgery, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

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ABSTRACT

Core facilities have a ubiquitous and increasingly valuable presence at research institutions. Although many shared cores were originally created to provide routine services and access to complex and expensive instrumentation for the research community, they are frequently called upon by investigators to design protocols and procedures to help answer complex research questions. For instance, shared microscopy resources are evolving from providing access to and training on complex imaging instruments to developing detailed innovative protocols and experimental strategies, including sample preparation techniques, staining, complex imaging parameters, and high-level image analyses. These approaches require close intellectual collaboration between core staff and research investigators to formulate and coordinate plans for protocol development suited to the research question. Herein, we provide an example of such coordinated collaboration between a shared microscopy facility and a team of scientists and clinician-investigators to approach a complex multiprobe immunostaining, imaging, and image analysis project investigating the tumor microenvironment from human breast cancer samples. Our hope is that this example may be used to convey to institute administrators the critical importance of the intellectual contributions of the scientific staff in core facilities to research endeavors.

ADDRESS CORRESPONDENCE TO: Douglas J. Taatjes, Department of Pathology and Laboratory Medicine, Microscopy Imaging Center, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA (E-mail: douglas.taatjes@med.uvm.edu).

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INTRODUCTION

Microscopy-based core facilities typically exist in one of two operational model structures: (1) “User Laboratories,” which provide access to multiple complex microscopy-based instruments and where facility staff advise investigators on the proper choice of the imaging system to use for their research and train them to use the instruments properly, and (2) “All-Inclusive Services,” where, in addition to the

services provided in the “User Laboratory” model, the staff now design, troubleshoot, and perform experiments and complex image analyses.[\[1\]](#) The Microscopy Imaging Center (MIC) in the Larner College of Medicine at the University of Vermont (UVM) is a core facility established in 1994 essentially as a “User Laboratory.” Originally consisting of a transmission electron microscope, a confocal scanning laser microscope, and an image analysis system, the MIC has evolved over the years into an “All-Inclusive Services” model core imaging facility to meet the diverse and expanding needs of the research base at the university, the medical center, and surrounding region. Much of the MIC staff activity revolves around providing consultation, experimental design, performing complex imaging-based experiments, training users in rapidly evolving immunohistochemical (IHC) protocols and use of the complex imaging systems, and providing image analysis and interpretation services to our diverse client base. These are hallmarks illustrating the value of academic core facilities as repositories of detailed knowledge in a wide variety of technical areas and how they may be best utilized to address individual research problems. Indeed, such shared core facilities have established a ubiquitous presence in research-intensive academic environments, with financial support often provided by the institution and various extramural granting mechanisms.[\[1\],\[2\],\[3\],\[4\],\[5\],\[6\],\[7\],\[8\]](#) Sadly, even though core facilities are viewed as an integral component to the success of institutional research endeavors, it is often difficult to convince clients of the importance of citing the contributions of core facilities as well as their funding sources (for example, instrumentation grants) in the acknowledgments section of published manuscripts.

Although they operate under a variety of business models, cores typically employ a “fee-for-service” cost recovery mechanism to generate revenue necessary to offset costs, including staff salaries and benefits, instrument service contracts, supplies, and even space charges.[\[2\],\[3\]](#) Often, the overarching goal from an administrative view is for core facility budgets to approach a “deficit neutral” annual outcome whenever possible. This goal is usually an unrealistic consideration, as it is difficult for most academic core facilities to balance staffing and overall operating costs with a potentially finite number of clients charged reasonable service rates. This leads to one of the laments often heard from core facility personnel: the idea that administrators responsible for core facility budgets keep a sharp eye on deficits to the detriment of not fully appreciating the degree of intellectual and scientific contributions shared resources offer to the overall research atmosphere. In this scenario, cores are sometimes perceived as mere “service centers” providing basic resources and

laboratory services for paying clients. Thus, core facility personnel struggle with ways to convey the crucial role that core facilities play in the overall research mission of an academic institution.

Certainly, there are abundant examples of the intellectual contributions of core facilities to research protocol development, and as described by Hogue-Angeletti et al[9] in reference to core facilities, “Development of new research opportunities may require trying different experimental approaches, testing and choosing among alternatives, taking risks.” Herein, we present a recent example of this concept, illustrating the critical importance of the combined knowledge present in core facilities as a resource to assist in a collaborative manner with a research group when presented with a complicated and multifaceted tissue staining and imaging requirement.

MATERIALS AND METHODS

Human tissue specimens were approved for research use having received UVM Institutional Review Board approval (No. CHRMS 15-629) and UVM Cancer Center Protocol Review and Monitoring Committee approval (No. VCC 1513). A clinical database search of UVM Medical Center records was performed to identify potential specimens of breast (normal, atypical ductal hyperplasia [ADH], ductal carcinoma in situ [DCIS], invasive ductal carcinoma [IDC]), and normal tissue controls (kidney, liver, and tonsil). Hematoxylin and eosin (H&E)-stained slides were retrieved for candidate samples and reviewed by a histopathologist to confirm project utility. Fresh H&E slides were prepared from formalin-fixed, paraffin-embedded (FFPE) tissue samples (collected between the years 2000 and 2015) confirmed for investigation. In the case of breast specimens, tumor regions were ink-marked on the H&Es by the histopathologist and used to guide tissue microarray (TMA) preparation. Using a Beecher Instruments Manual Tissue Arrayer (Estigen OÜ, Tartu, Estonia), 2-mm core punches of breast tissue (normal, ADH, DCIS, IDC, and adjacent stromal tissues) were sampled from FFPE blocks made with patient surgical specimens to construct TMA blocks with up to 30 patient samples per TMA. Then, 4- μ M tissue sections of TMA and normal kidney/liver/tonsil FFPE blocks were slide mounted and heated at 60°C for additional H&E staining (TMAs) and stored at 4°C, or unheated tissue sections were stored at –20°C prior to IHC/immunofluorescence (IF) procedures (all samples). To avoid using precious and expensive patient breast cancer TMAs for protocol development, human tonsil paraffin sections were used as a positive sample for the planned antibody panel.

Tissue staining protocols

Iterative IF

Iterative IF stainings were performed via staining, imaging, stripping, and restaining the same tissue sample for all antigens of interest. These methods will only be briefly described, as they were abandoned in favor of the final detailed methods provided below. Sections on TMA slides were conventionally deparaffinized through treatment with xylenes and graded alcohols through distilled water. Antigen retrieval was then performed with DAKO Target Retrieval solution (Agilent Technologies) for 20 minutes at 95°C in an antigen decloaking chamber. Sections were then treated with 10% normal goat serum for 1 hour at room temperature, followed by incubation with primary antibody overnight at room temperature ([Table 1](#)). Following buffer rinses, the sections were incubated with appropriate goat anti-mouse or goat anti-rabbit Alexa 488 conjugated secondary antibody (Thermo Fisher Scientific) diluted 1:500 in 1% bovine serum albumin for 1 hour at room temperature, rinsed and stained with 4',6-diamidino-2-phenylindole (DAPI) (10 mg/mL), and finally rinsed and mounted using aqueous mounting media. Slides were imaged using a Nikon A1R high-definition (HD) confocal microscope (Nikon Instruments Inc.) with a 40× objective lens (Plan Fluor 40× numerical aperture (NA) 1.3). Coverslips were then removed by soaking in water with agitation. A thin razor blade was used to loosen the corners of the coverslip, followed by agitating in water until the coverslip floated off. Following coverslip removal, slides were incubated with glycine 20% sodium dodecyl sulfate pH 2 solution at 50°C to strip the previous round of staining. Slides were washed abundantly with phosphate buffered saline/0.5% Tween 20, and the staining and imaging process was repeated for the next target. The iterative process was then continued until all targeted antigens were stained and imaged.

Table 1

Compiled list of reagents used for the three protocols discussed, Iterative IHC, Iterative IF, and Multiplex IF. NA, not available.

Reagent	Vendor	Product number	Concentration	Dilution	Diluent	Iterative IHC	Iterative IF	Multiplex IF
anti-CD34	Leica	PA0212	10 mg/ml	neat		X	X	X

anti- α smooth muscle actin	Leica	PA0943	10 mg/ml	neat		X	X	X
anti-CD68	Leica	PA0273	10 mg/ml	neat		X	X	X
anti- Vimentin	Agilent	IR630	NA	neat		X	X	
anti- TP63	Cell Signaling	D9L7L	NA	1:50	Opal 1X Amp			X
anti-CD3	Leica	PA0553	10 mg/ml	neat		X	X	X
anti-CD90	Abcam	133350		1:100	TBS 1% BSA			X
Goat anti- mouse Alexa 488	Jackson ImmunoRe search	115-545- 003		1:500	1% BSA		X	
AEC substrate kit	Vector	SK-4200				X		
VectaMoun t AQ	Vector	H-5501-60				X		
ImmPRESS anti-mouse IgG kit	Vector	MP-7452- 15				X		
Opal 7- Color Multiplex kit	Akoya Bioscience s	NEL82100 1KT						X
Opal 520	Akoya Bioscience s	Opal kit component		1:200				X
Opal 540	Akoya Bioscience s	Opal kit component		1:100				X

Opal 570	Akoya Biosciences	Opal kit component		1:200				X
Opal 620	Akoya Biosciences	Opal kit component		1:500				X
Opal 650	Akoya Biosciences	Opal kit component		1:1000				X
Opal 690	Akoya Biosciences	Opal kit component		1:100				X
ProLong Gold Antifade	Invitrogen	P36930						X

Iterative immunohistochemistry

Iterative immunohistochemical stainings were performed essentially as described by Tsujikawa et al.[\[10\]](#) and Glass et al.[\[11\]](#) FFPE sections on TMA slides were conventionally deparaffinized through treatment with xylenes and graded alcohols through distilled water. Antigen retrieval was then performed with DAKO Target Retrieval solution for 10 to 20 minutes at 95°C, depending on the primary antibody. Endogenous peroxidase was quenched by incubating the sections with 3% peroxide in methanol for 10 minutes at room temperature with agitation. Slides were rinsed, and sections were then treated with 2.5% normal goat serum/0.1% Tween 20 for 20 minutes at room temperature, followed by incubation with primary antibody for 1 hour at room temperature ([Table 1](#)). Following buffer rinses, sections were incubated with polymer (anti-mouse and anti-rabbit IgG-HRP-conjugated secondary polymers; Vector ImmPRESS kit; Vector Labs) for 30 minutes at room temperature and then rinsed and incubated with AEC (3-amino-9-ethylcarbazole) for 5 to 15 minutes, depending on the primary antibody target. Sections were rinsed and counter stained with hematoxylin for 1 minute, dipped in lithium carbonate for 2 minutes, and mounted with VectaMount AQ (Vector Labs). Slides were imaged on a Leica-Aperio VERSA 8 whole-slide imager (Leica Biosystems) with a 40× objective lens (HC Plan Fluor; NA 0.85). Coverslips were then removed by soaking in water with agitation. A thin razor blade was used to loosen the corners of the coverslip, followed by agitating in water until the coverslip floated off. Following coverslip removal, AEC staining was removed by rinsing in water

and graded alcohols, terminating in 95% ethanol until AEC stain was washed out. Slides were next rinsed in graded alcohols through to water. The procedure was then repeated starting with antigen retrieval, and the slides were stained for the next target of interest until all targets had been stained and imaged.

Enhanced multiplex staining with Opal 7-Color IF kit

Human breast tissue biopsy samples and control tissues (kidney, liver, and tonsil) were stained with a custom-designed stromal panel using an Akoya Opal 7-Color Automation kit (NEL821001KT; Akoya Biosciences) on a Leica BOND RXm slide autostaining system (Leica Biosystems; software version 6). Antigenic targets of interest and corresponding Opal dye pairs are described in [Table 2](#). Immunostaining protocol steps were established and developed according to the Perkin Elmer user manual titled, “Opal 4-Color and 7-Color Automation IHC Kits for Leica Biosystems BOND RXm System Software version 4.0.” Briefly, each antibody used in the staining panel was optimized individually by performing a dilution series on positive control tissue (tonsil or breast). Once the optimal antibody concentration was identified, antibodies were paired with an Opal dye based on dye intensity and expected antigen abundance. Antibodies labeling antigens expected to be less abundant in the tissue of interest were paired with brighter Opal dyes and stained earlier in the iterative process than more-abundant antigens. Once staining order and dye pairings were established, dye concentrations were adjusted to maximize the specific signal for each antibody and to provide flexibility in adjusting laser intensities.

Table 2		
List of antibodies and Opal dye pairs used for spectral imaging.		
Target	Cell Type	Opal dye
CD3	Pan T- cell	Opal 520
CD68	Macrophage	Opal 540
CD90/Thy1	Mesenchymal stromal cells	Opal 570
CD34	Hematopoietic stem cells, Mesenchymal stem cells	Opal 620
α SMA	Stromal fibroblasts	Opal 650
TP63	Basal/myoepithelial cells	Opal 690

Confocal large-scale imaging

Imaging was performed with a Nikon A1R HD laser scanning confocal microscope equipped with an A1-DUS spectral detection unit and using 405-, 488-, 561-, and 640-nm laser lines. Image scans were acquired with a 40× CFI Plan Apochromat I (NA 0.95) objective lens using the large image scan function under the “Acquire” menu at a resolution of 0.3 mm/pixel. The left, right, top, and bottom limits were defined (up to 1,000 individual 40× images). Spectral detection covering the wavelength range from 420 to 740 nm was accomplished with 10-nm diffraction gradient bandwidth passes using a galvanometric scanner, and images were acquired with a 32-channel photomultiplier tube. Images were then stitched into one large image scan using Nikon NIS Elements software (version 5.2.1; Nikon Instruments Inc.). Unfortunately, the resulting file sizes of the tiled and stitched spectrally acquired images were too large to be unmixed in NIS Elements, so an alternative approach was developed requiring the tiled image to be split, spectrally unmixed as separate tiles, and then finally restitched.

ROI or TMA core imaging

A routine was scripted in the Nikon NIS Elements JOBS software module, which allowed the custom creation of an image acquisition workflow with graphical programming, to acquire a 4× (Plan Apo λ; NA 0.20) overview image at 256 × 256 pixel resolution with 405-nm (imaging DAPI signal) and 640-nm excitation (imaging α-smooth muscle actin [α-SMA] signal). This low-resolution overview scan was necessary because, due to either the number of tissue cores per slide or large sections (~2 mm) of tissue per slide or the size of the resulting image, the system quickly ran out of memory at higher-resolution scans. The low-resolution image was then used to define ROIs for imaging at 40× (CFI Plan Apochromat Lambda; NA 0.95). A 40× dry objective lens was ultimately chosen for imaging, as after many attempts to develop a slide oiling technique that was reliable, it was determined that the autofocus failure rate over such a large slide area was approximately 40%. After switching to the air interface objective, the focus failure rate fell below 5%, with no measurable differences in the resulting data sets with the quantification methods used. ROI images were acquired with the galvanometric scanner and 10-nm diffraction gradient spectral detection and saved individually in ND2 format as scripted by the JOBS protocol. Laser power and high-voltage settings were conserved across all images in the data set.

Spectral unmixing

Individual spectral libraries were created by immunostaining sections from human tonsil with anti- α -SMA antibody (Leica Biosystems; product No. PA0943) using each of the secondary antibody Opal dyes contained in the Akoya Opal 7-Color Automation kit (Opals 520, 540, 570, 620, 650, and 690). The immunostainings were performed on individual sections (anti- α -SMA and one Opal dye) to obtain spectral emission curves for each of the Opal dyes using laser power settings matching those used for the final samples. These spectral libraries were ultimately used to spectrally unmix raw images for the test samples using the linear unmixing algorithm provided in the NIS Elements software.

Image analysis

Indica Labs HALO

The enhanced multiplex Opal-7 assay spectrally unmixed confocal images were analyzed and quantified using HALO and HALO AI analysis packages (Indica Labs). Costaining of antibodies was assessed with HALO module “HighPlex FL” (version 3.2.1) to perform nuclear segmentation and phenotyping of the cells. A threshold for nuclear stain represented by DAPI signal was set to delimit the nuclear area. Membrane and cytoplasmic detection was defined with a maximum cytoplasmic radius of 3 units and membrane segmentation aggressiveness set to 0.9. Nuclear detection was defined with a contrast threshold of 0.5, minimum nuclear intensity of 0.0002, maximum image brightness of 1, and nuclear segmentation aggressiveness of 0.65. Thresholding was completed for all 6 Opal dyes, indicating staining for the chosen antigen. Thresholds were established from secondary antibody control incubations to account for any background staining. Additionally, the subcellular localization of individual antigens was considered for phenotyping and cell segmentation. Multiple coexpression immunostaining phenotypes were selected and defined as staining for multiple antigens within the same cell (eg, TP63/ α -SMA, CD68/CD34, etc.).

The total number of immunostained cells and coexpression phenotypes were analyzed and characterized throughout the entire tissue section, individual ROI images, or individual TMA cores. Raw object data obtained from the HALO Highplex FL analysis were exported and further analyzed using custom R (R Core Team, 2020) scripts.[\[12\]](#)

HALO AI

Using a custom-trained HALO AI DenseNet classifier, ROI images were classified as either tumor or stroma. The tumor regions were further designated as normal, DCIS,

IDC, or ADH based on the pathologist's annotations on the H&E-stained serial section slide images. The above-described HALO Highplex FL was used to perform the analysis within these classified regions.

Improperly segmented cells were removed using R-script to identify cell size outliers.

Spatial analysis

Spatial analysis was performed on the HALO Highplex results using the Proximity Analysis algorithm from the Spatial Analysis module within HALO. The distance between cells positive for each marker and cells that were copositive for TP63 and a-SMA as surrogate for epithelial boundary was measured and plotted as a spatial plot or as a histogram.

RESULTS

The professional scientific staff in the MIC core was approached to assist with a very large and complicated project involving the staining, imaging, and image analysis of whole breast cancer TMAs as well as large DCIS breast sample slides using a custom-designed 6-antibody panel for identification of tissue stromal antigens plus a nuclear stain. The core was charged with designing, developing, and troubleshooting the antibody staining protocols as well as performing the microscopy imaging and subsequent image analyses. At that time, given the instrumentation available in the core, it was decided to approach this project via iterative multiplex immunostaining and automated microscopy imaging using a whole slide imager (not shown). Both fluorescence- and enzyme-based methods were explored and developed simultaneously. Even though the iterative multiplex immunostaining was relatively successful, it quickly became apparent that the subsequent alignment of the resulting temporal images acquired by whole slide imaging would be troublesome and time-consuming, leading us to seek alternative methods.

We determined that the key to overcoming these technical issues was to add new capabilities to existing equipment as well as acquire new and needed instrumentation and software. Fortunately, at this time, the NIGMS-funded Northern New England Clinical Translational Research Network (NNE-CTR) provided support to (1) upgrade a Nikon A1R HD confocal microscope with a spectral detector, (2) purchase a Leica BOND RXm autostainer, and (3) purchase Indica Labs HALO image analysis software. At the same time, we received a Larner College of Medicine Shared Instrumentation grant providing funds to add JOBS software modules to the Nikon A1R confocal microscope NIS Elements software, providing the ability to create automated routines

for complex image acquisitions. With these new tools in hand, we completely redesigned our experimental protocols, leveraging the spectral detector together with an Opal 7-color kit for multiplex immunostaining.

Using paraffin-embedded human tonsil arrays as a test sample to preserve valuable breast tumor TMAs, MIC staff working together with the research group developed the detailed protocol shown in [Figure 1](#) for automated tissue section immunostaining, confocal microscopy spectral image acquisition,[\[13\]](#),[\[14\]](#) and robust semiautomated image analysis using HALO software. To perform confocal microscopy spectral imaging, reference emission profiles for each of the Opal dyes were acquired using sections from tonsil stained with a-SMA antibody, followed by each individual dye ([Figure 2](#)). Once the reference spectra were collected, they could then be used for spectral unmixing of a section stained with the 6-antibody panel and DAPI. [Figure 3](#) shows the original unprocessed acquisition images from an Opal 7-color kit-stained tonsil section together with the same images following application of the linear unmixing algorithm. Note the clear separation apparent between cells stained with the 6 different antibodies ([Figure 3B](#)).

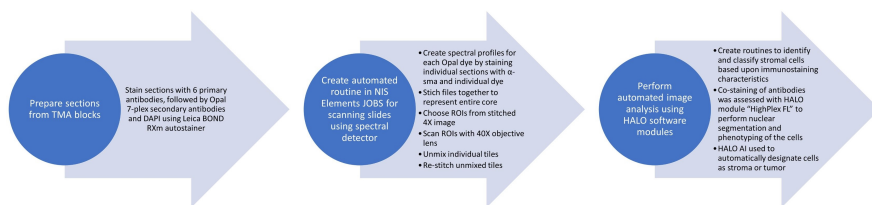
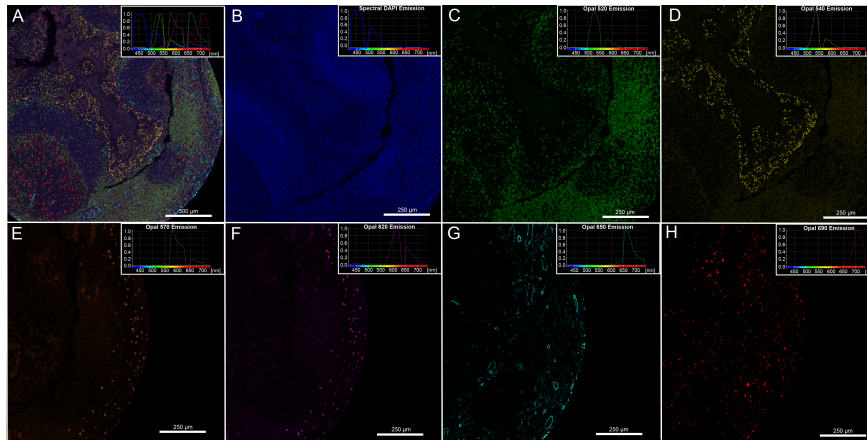


Figure 1

Diagrammatic representation of the steps involved in the multiplex staining and spectral imaging and analysis protocols developed by core imaging staff working with a research group.

**Figure 2**

Confocal spectral images and spectral dye emission signatures (*Insets*) acquired by staining sections from human tonsil with an antibody against α -SMA followed by one of each of the 6 Opal dyes. (A) All 6 of the dyes; (B) DAPI alone; (C) Opal 520; (D) Opal 540; (E) Opal 570; (F) Opal 620; (G) Opal 650; (H) Opal 690. The inset in each panel shows the spectral emission profile for each of the Opal dyes. Thresholding was applied to the images for visualization purposes.

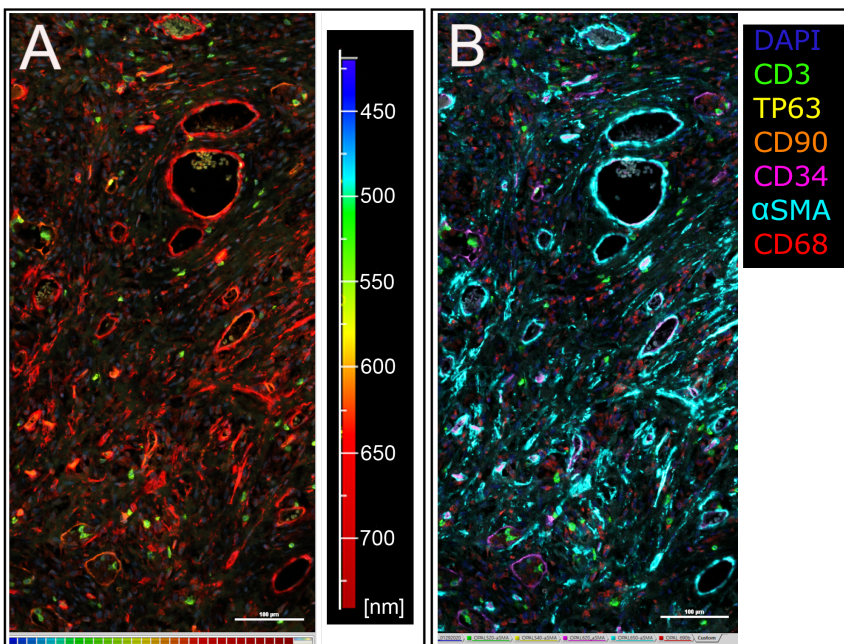


Figure 3

Images of human tonsil stained with a custom-designed antibody panel containing 6 antigen targets labeled with Opal dyes plus DAPI. (A) Composite raw spectral image with signal shown in 10-nm sweeps through the visible spectrum. The color bar to the right of the panel indicates the wavelength corresponding to the colors in the image. (B) Image after spectral unmixing with experimentally obtained spectral libraries. Each image pixel was assigned a color corresponding to the Opal dyes used in the staining panel as indicated in the color key to the right of the image. Thresholding was applied to the images for visualization purposes.

Once the proof-of-concept experiments were completed with the tonsil sections, the same protocol was used on TMAs from the breast cancer cohort ([Figure 4](#)). We have developed a large cohort of breast cancer samples into TMAs. Each patient in the TMA is represented by 3 individual cores: the first taken at the tumor stroma boundary, followed by a second adjacent core taken 2 mm away from the first into stroma, and then a third 2 mm away from the second core. These cores represent the tumor microenvironment as a measure of distance from the tumor. We developed a multiplex panel of markers as described in [Table 2](#) that facilitates identification of multiple cell types in stroma surrounding the tumor. [Figure 5](#) shows a representative image from one of the breast TMA cores in which multiplex IF using Opal dyes was performed to identify multiple cell types in tissue epithelium and stroma ([Figure 5B](#)). Cells were determined to be positive for a given marker based on stain intensity threshold values.

The threshold values were determined visually by comparing dye intensities in stained tissue sections to secondary control tissue sections. The individual TMA core was annotated by a pathologist on a serial section stained with H&E ([Figure 5A](#)) and used as guidance to mark benign, stroma, and other areas such as adipose and vascular tissue on sections stained with multiplex staining ([Figure 5C](#)) using HALO 3.2 image analysis software. The annotated multiplex images were further analyzed using Highplex IF analysis module within HALO to perform cellular segmentation to identify nuclei and cells stained with individual markers ([Figure 5D](#)). The object data obtained from HALO analysis was used to calculate cell frequencies/densities within individual annotated regions as well as the entire core to identify distributions of different single- or dual-expressing cells within epithelium and surrounding stroma using R scripts ([Figure 5E](#)). The boxed region in [Figure 5B](#) is shown at higher magnification in [Figure 6](#), revealing examples of some single positive and copositive cells identified in our study. Highlighted cells include singly stained CD-68-positive macrophages (arrowheads in [Figure 6](#)) and myoepithelial cells lining the ducts showing α -SMA and TP63 coexpressing cells (arrows in [Figure 6](#)).

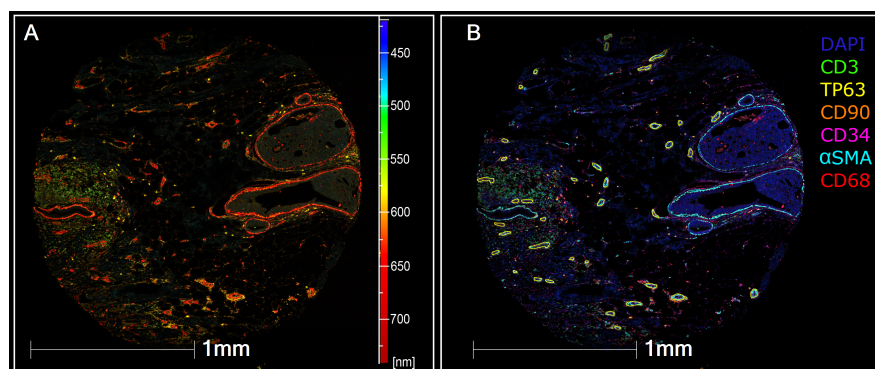
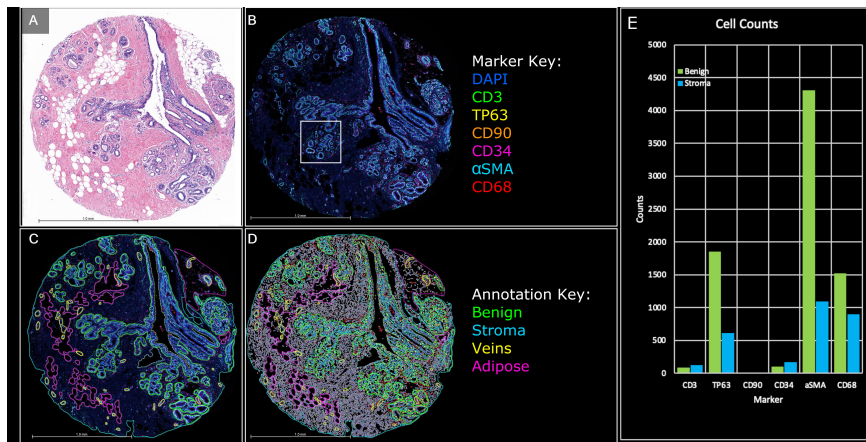
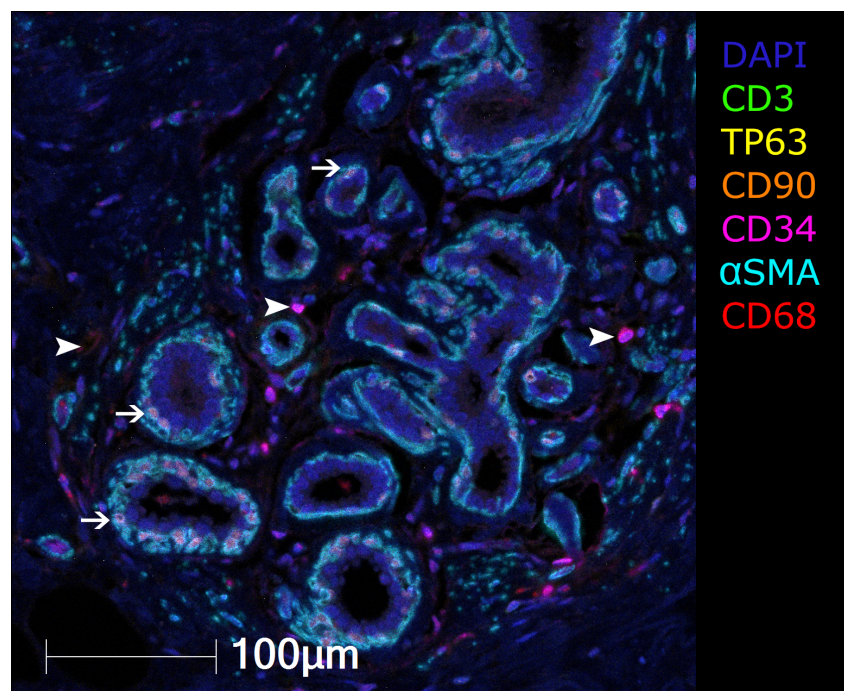


Figure 4

Images of human breast TMA stained with a custom-designed antibody panel containing 6 antigen targets labeled with Opal dyes plus DAPI. (A) Composite raw spectral image with signal shown in 10-nm sweeps through the visible spectrum. The color bar to the right of the panel indicates the wavelength corresponding to the colors in the image. (B) Image after spectral unmixing with experimentally obtained spectral libraries. Each image pixel was assigned a color corresponding to the Opal dyes used in the staining panel as indicated in the color key to the right of the image. Thresholding was applied to the images for visualization purposes.

**Figure 5**

Representative image showing breast cancer TMA core with multiplex IF staining using Opal 7-color kit. (A) Serial section with H&E staining to demonstrate tissue pathology. (B) Multiplex IF image showing staining with 6 antibodies in single section. Nuclei are stained with DAPI. (C) Multiplex IF image showing different annotated regions to identify epithelium and stroma within each core. (D) Markup image after HALO analysis using appropriate nuclear and cellular segmentation algorithms. The micrograph shows distribution of individually stained cells within different annotated regions of the TMA core. (E) Example of graphical representation of the object data obtained after HALO image analysis showing distribution of single-stained cells within benign epithelium and stromal regions expressing specific antigens. Thresholding was applied to the images for visualization purposes.

**Figure 6**

“Zoomed in” boxed region of interest of breast TMA core shown in Figure 5B. Immunostaining patterns of individual cells can now be discerned at this higher magnification. Each image pixel was assigned a color corresponding to the Opal dyes used in the staining panel as indicated in the color key to the right of the image. Thresholding was applied to the images for visualization purposes.

The data showing detailed comparative analyses of multiple cellular phenotypes within individual patient samples as well as comparison between individual patient samples is currently being collected, and the results comprising the large overall project will be presented in a subsequent manuscript. Here, we only describe how the newly developed protocols provided the means to address this complex project. Interestingly, a rough estimate of the hands-on staff time invested to complete the initial iterative immunostaining and imaging method, either enzymatic or fluorescent, was approximately 8 working days (64 hours). To complete the same number of antibody stains with the enhanced multiplex IF Opal 7-color kit required approximately 5 to 6 hours of hands-on staff time; an additional 20 hours of automated staining and imaging time was also required per slide (13 to 14 hours of automated staining time for a batch of 10 slides and 6 to 7 hours of confocal imaging and spectral unmixing per slide). Using an internal grant from the university’s Office of the Vice President for Research, we purchased high-capacity NAS file storage devices and installed 10G fiber lines to

enhance file transfer between the different systems during image acquisition, processing, and analysis, providing additional time savings when working with the very large data sets.

DISCUSSION

Academic core facilities are assuming an increasingly important role in academic research settings, as instrumentation and experimental protocols evolve to ever higher levels of complexity. For instance, the complexity of state-of-the-art biomedical research methods such as spatial transcriptomics[15],[16],[17],[18],[19] requires engaging in “Team Science,” leveraging the expertise from many different cores, disciplines, and individuals. In this light, the close collaboration and sharing of expertise between bench research scientists and core facility staff in the development of innovative cutting-edge protocols will likely represent the “norm” as scientific approaches advance at break-neck speed.[20] We believe this concept is well illustrated in our current manuscript. Although the goals of the project were well conceived, the complex technical issues confronted at the outset were not fully appreciated. Several multiplex immunostaining protocols were available at the time, and we began to use them for this project:[10],[11],[21],[22] however, precise registration of the images acquired following each round of immunostaining posed a complex problem preventing reproducible quantitative analyses. After much consideration and deliberation, we determined that a multiplex-fluorescence approach using overlay of images acquired from a single session on a confocal microscope available in the MIC would provide a more ideal solution. To allow this approach required several technical improvements to the instrument and software capabilities then available in the MIC: (1) the addition of a spectral detector unit and associated software to the confocal microscope; (2) acquisition of a tissue slide autostainer to automate and assure reproducibility of the complex staining protocols; and (3) acquisition of state-of-the-art high-power computer workstations, image analysis software, and high-speed data transfer lines to process and handle the very large data sets obtained via this methodology. Working in partnerships with members of the NNE-CTR, the Larner College of Medicine Dean’s Office, and the Office of the Vice President of Research at the university, we were able to justify and then obtain the necessary funds for the instrument upgrades and new purchases.

Once these advanced hardware and software components were in place, detailed protocols were developed and tested. The resulting protocol developed by the combined efforts of the MIC core staff and the research laboratory personnel using

human tonsil paraffin sections as a positive control tissue was successfully implemented for the breast cancer paraffin TMAs. As this manuscript is being prepared, further breast cancer TMAs are being stained, imaged, and analyzed using the designed protocol, with the overall findings to be presented in a subsequent manuscript. Lacking the funds mentioned above to upgrade and add additional equipment and software certainly would have resulted in significant delays in progress of the project and perhaps even to seeking external solutions. However, unlike relatively straightforward commercial-based solutions such as sequencing, this project consisted of multiple stages, including sample acquisition and processing, antibody panel selection, testing the order of antibody addition together with matching to the optimal Opal dye, spectral database construction, spectral imaging, and complex image analyses. All of these steps required daily close interaction between core staff and project researchers, which would not have been possible if the project were to have been (or even could have been) “outsourced.” Besides, from the perspective of the MIC core, a further goal of this project was to develop a streamlined process for multiplex immunostaining and subsequent image analysis utilizing instrumentation and software available in the core to be offered as a new service for our clients. Indeed, as of this writing, several research groups at our institute have contracted with the MIC core to perform our newly developed immunostaining and analysis protocol for their research projects, enhancing their research by increasing the number of antigens localized in a tissue section together with analyzing the spatial locations of the cells expressing different antigen combinations.

The success of this project required close collaboration between multiple personnel from the core facility and the research laboratory. Because the designed innovation required more effort and time than normally available for core personnel, the research group covered their time and effort for this project by absorbing a percentage of their efforts on the relevant research grant. This was a key decision allowing the project to proceed at a rapid pace. Certainly, neither the core staff nor the research group working alone would likely have resulted in a positive and successful outcome. Moreover, the degree of autonomy of the MIC director to approve staff time to pursue innovative solutions to client-posed complex research problems played a large supporting role in the ultimate success of this endeavor. During this time, routine core duties were still required, and timelines for handling client samples and services were maintained. This was partially accomplished by staining, imaging, and analyzing the samples oftentimes at core “off-hours” (evenings and weekends). Likewise, instrumentation and software acquired for this project provided automated

immunostaining, image acquisition, and image analysis, greatly accelerating all of these steps and thus allowing sufficient access for other client projects. This situation may not be applicable to all core facilities, but perhaps our example described herein may serve as a template for others striving to expand core services to address the burgeoning complexities of client requests.

The intellectual and problem-solving contributions of the MIC staff members during the conceptualization and implementation of this imaging project underscore the overall critical importance of core facilities to the research success of an institution. [20] The investment in cell imaging capabilities at our institute has been instrumental in securing extramural support, including a National Cancer Institute Program Project grant on cancer-compromised epigenetic control and a National Cancer Institute Consortium grant to address mechanistic and clinically informative crosstalk between breast cancer and the tumor microenvironment. Moreover, in addition to providing “routine” technical services often not available in individual investigator laboratories, core facilities can serve as a hub for efforts to improve biomedical research rigor and reproducibility by providing proven standardized protocols and methodologies. [23], [24], [25]

As mentioned above, the collaboration of multiple core facilities together with research groups to provide state-of-the-art yet individualized innovative complex experimental capabilities, such as in the realm of “spatial multi-omics” (recently named one of “Seven Technologies to Watch in 2022” by *Nature* magazine [26]), likely represents the future for research institutions, highlighting again the critical value of core facilities to research success. [18] In this regard, our institute recently coalesced multiple shared resources (microscopy imaging, integrated genomics, flow cytometry, and proteomics) into the Center for Biomedical Shared Resources (CBSR). The CBSR will be occupying the entire first floor of a now under construction biomedical research building, with an open architectural design to promote cross-collaborations and innovative thinking among the various cores in the facility. For research institutions to continue to succeed, publishing high-quality and innovative results as well as garnering extramural funding, core facilities will need to continue to evolve from strictly service-oriented offerings to collaborative operations, merging the intellectual abilities of staff members with researchers to provide novel and innovative approaches to solve complex research questions. [27]

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